Biomarkers of Exposure to Airborne Criteria and Air Toxic Pollutants

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California Air Resources Board

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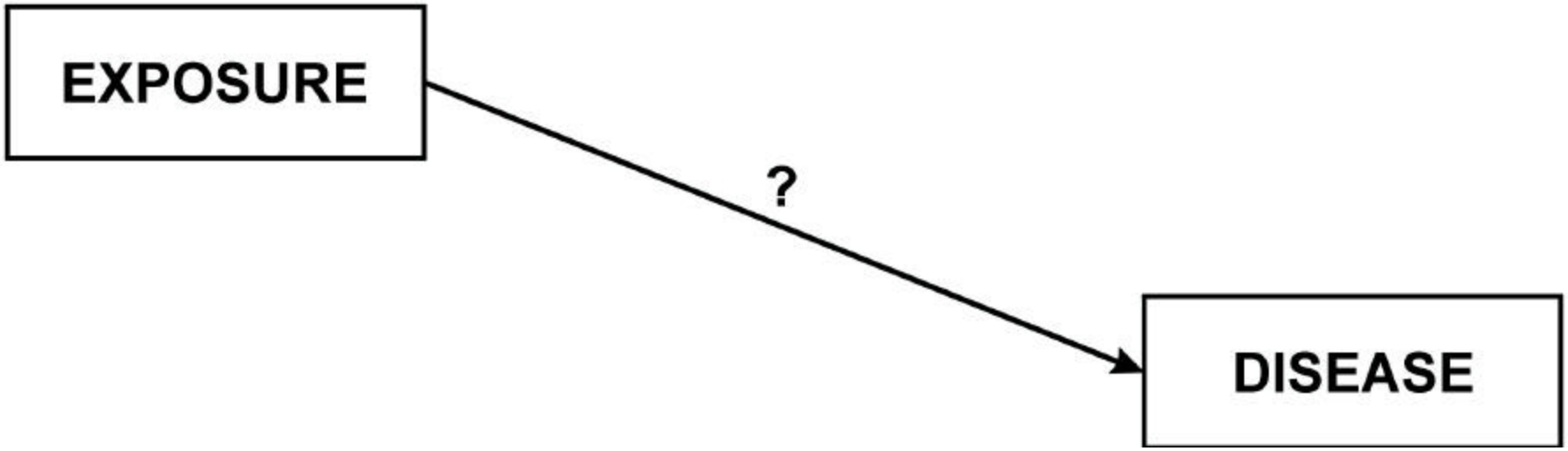
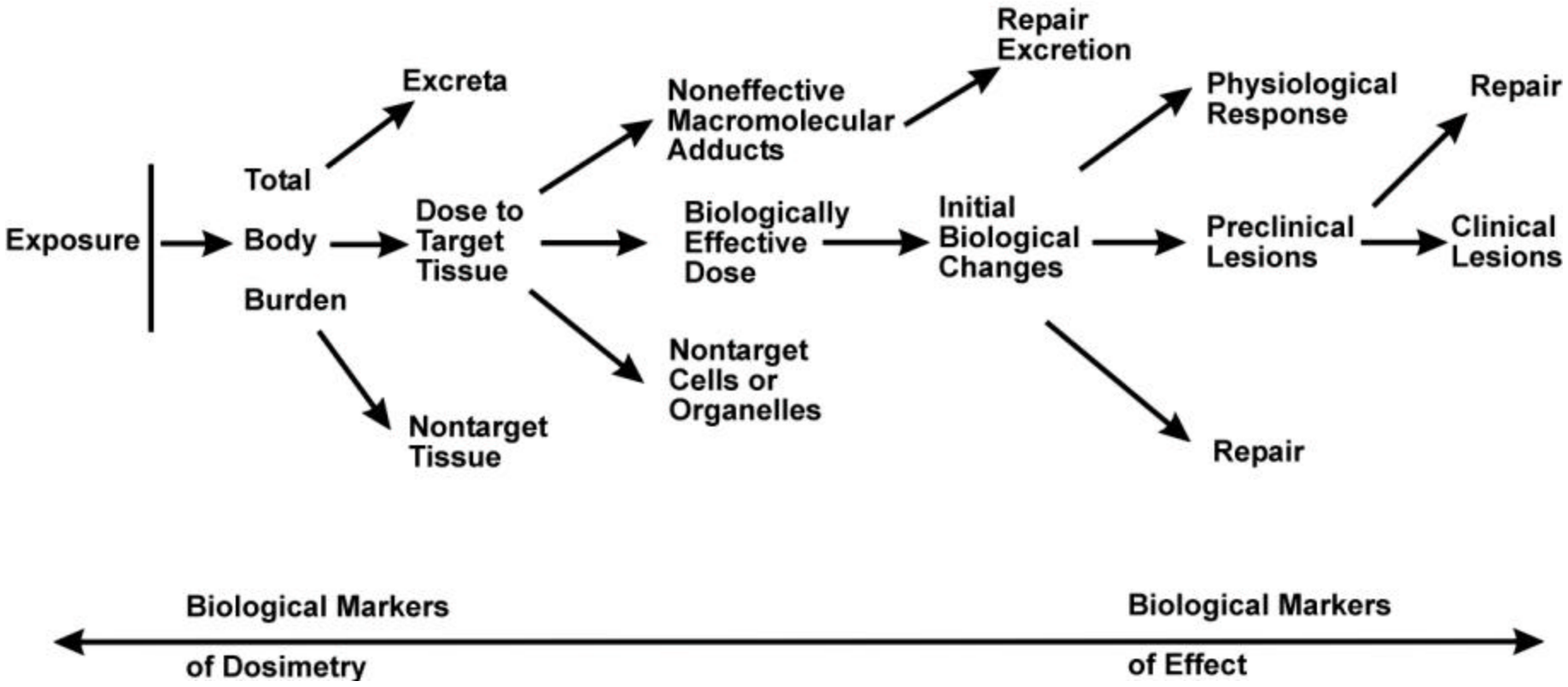
Use of Biological Markers to Reduce Uncertainties in Risk Assessment

To what extent does exposure to chemicals contribute to disease?



Biomarkers for Risk Assessment



Biological Marker of Exposure

An exogenous substance or its metabolite or the product of an interaction between a xenobiotic agent and some target molecule or cell that is measured in a compartment within an organism.

Ideal Biomarker of Exposure

- **Quantitatively relatable to prior exposure to specific chemical**
- **Quantitatively relatable to, or predictive of, later developing disease**

Sometimes, we only need a qualitative, yes/no answer.

Have you been drinking again?

Sometimes quantitation is required.

But I only had one beer!

Desirable Attributes of Biomarkers of Exposure

- Pollutant specific
- Available for analysis by noninvasive techniques
- Sensitive (detectable at trace concentrations)
- Inexpensive
- Integrates exposures from all media

Strategies to Relate Markers of Exposure to Prior Exposures

What information do we need to relate a biomarker of exposure to prior exposures?

If we want to be more quantitative, more information is required.

- **Rate of formation of biomarker**
- **Rate of formation of biomarker**

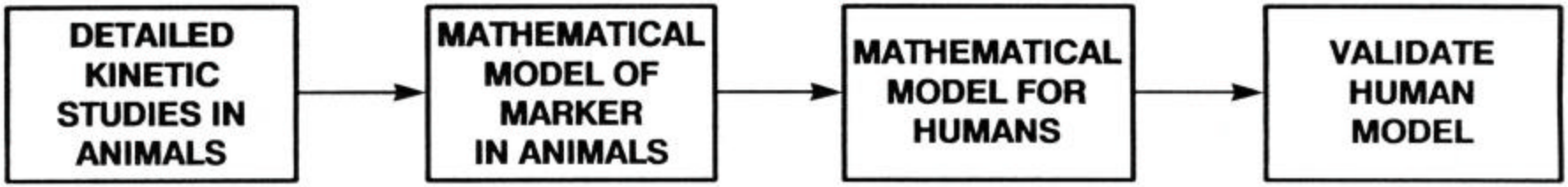
From this information, we can predict the steady-state concentrations of the marker following various exposure scenarios.

Mathematical Models

- If we have information on rate of formation and removal of a biomarker of exposure, and the factors that influence those rates, we can develop a mathematical model that will predict the concentration of the marker following various exposure regimens.

- The concentration of a biomarker cannot be used to indicate a unique exposure scenario, but can indicate the types of exposure regimens that would produce the indicated level of biomarker

Use of Animal Data on Kinetics of Biomarkers to Predict Kinetics in Humans



- PERCENT ABSORBED
- $T_{1/2}$ TO STEADY STATE
- $T_{1/2}$ FOR CLEARANCE
- EFFECT OF
  - EXPOSURE CONCENTRATION
  - EXPOSURE RATE
  - REPEATED EXPOSURES
  - ROUTE OF EXPOSURES

- ANIMAL TOXICOKINETIC DATA
- ANIMAL PHYSIOLOGICAL DATA

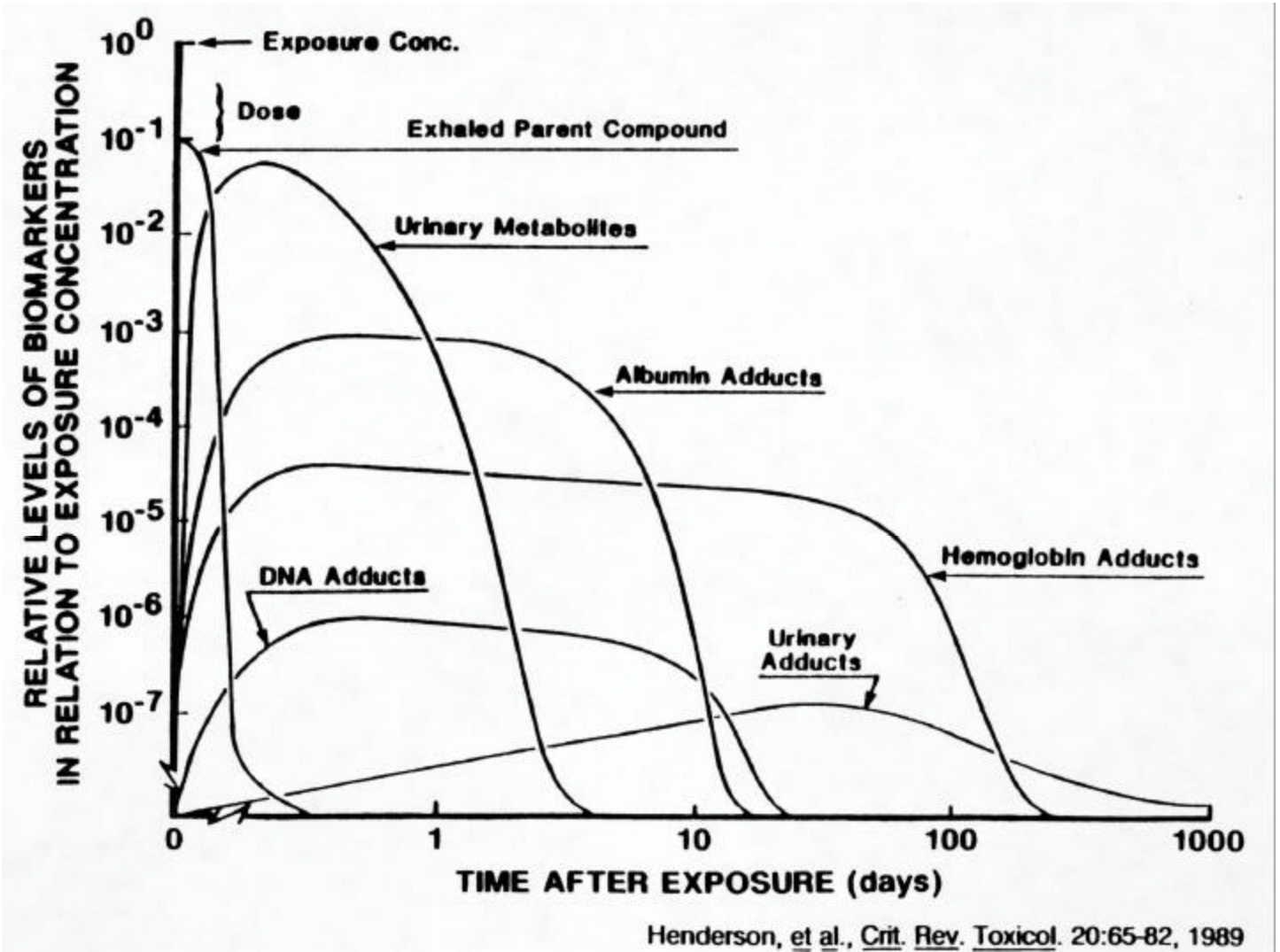
- HUMAN PHYSIOLOGICAL DATA
- HUMAN METABOLIC DATA IN VITRO

- LIMITED HUMAN DATA

A Second Strategy:

A battery of biomarkers of exposure with differing half-lives in the body may provide more information about prior exposures than a single biomarker.

Hypothetical Relationship Between "Biomarkers" and Time After Exposure



Henderson, et al., Crit. Rev. Toxicol. 20:65-82, 1989

Use of a Battery of Biomarkers to Determine Past Exposure

Marker	Marker $t_{1/2}$	Case I	Case II	Case III
A	minutes	+	+	--
B	hours	+++	+++	--
C	days	++	++	--
D	weeks	+	+++	++
E	months	+	+++	++

Case I - Recent exposure.
Case II - Ongoing exposure.
Case III - Exposures were several months ago; no recent exposure.

Marker? Disease

Strategies to Relate Markers of Exposure to Health Outcome

Need to know which markers can be associated with the disease outcome and the degree of the association. That is, given a certain level of a biomarker of exposure, what is the probability of getting the disease?

What information is required to relate markers of exposure to probability of health outcome?

- **Mechanism of disease induction**
- **Quantitative relationship between marker and probability of progression to adverse health effect**

Example:

Predicting cancer induction using DNA adducts as biomarkers

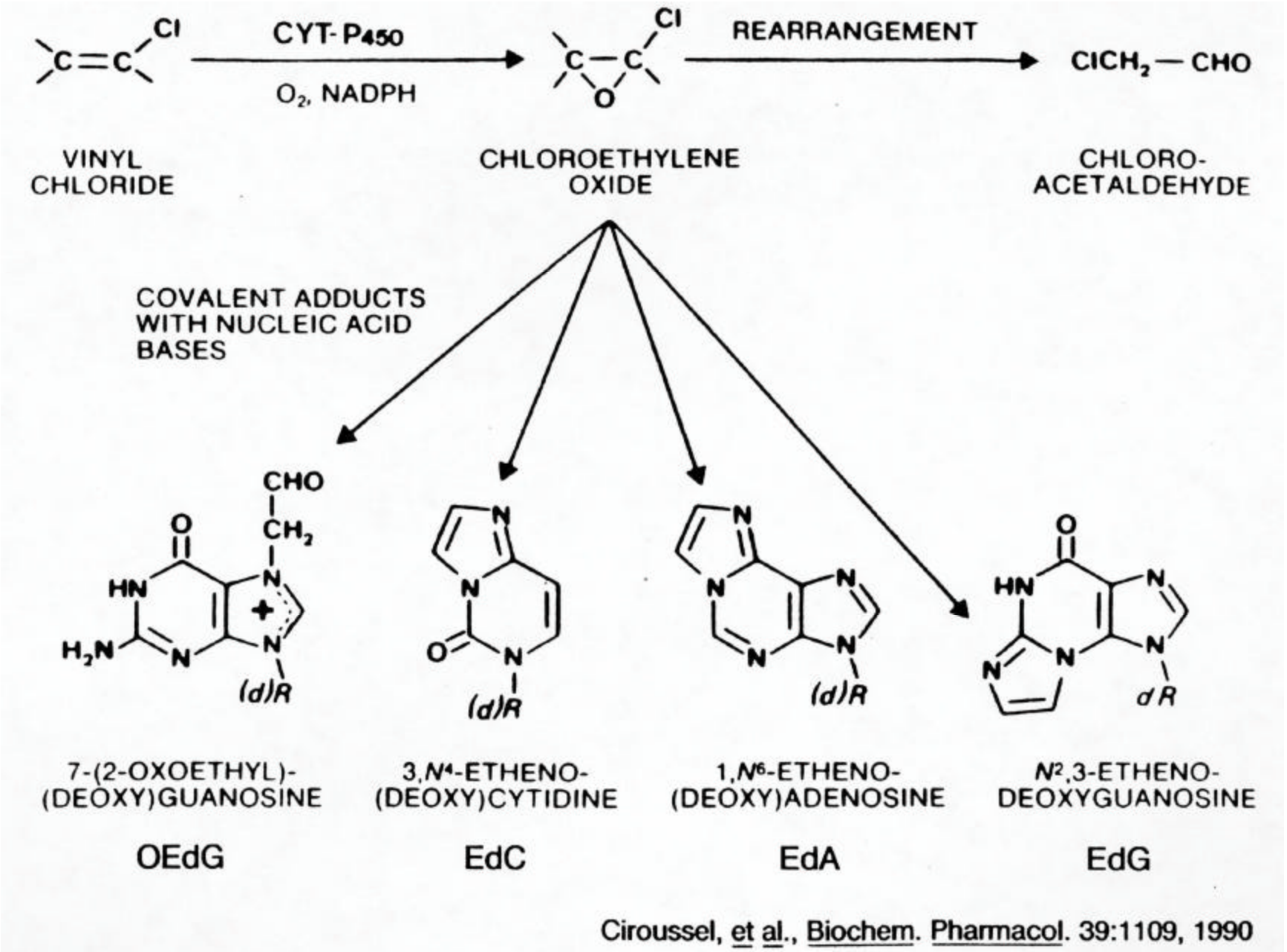
- **Identify DNA adducts that are formed by exposure to chemical**
- **Determine $t_{1/2}$ of adducts**
- **Determine mutagenic potential of adducts**
- **For adducts with long $t_{1/2}$ and mutagenic potential, determine if mutations induced by adduct are present in tumors induced by chemical**
- **In animals, develop pharmacodynamic models that describe a quantitative relationship between adduct levels and cancer induction**

Example: DNA adducts of vinyl chloride (VC)

VC causes liver cancer in humans and animals. In rats, preweanling rats are more susceptible than adults.

Swenberg et al., Conference of Relevance of Animal Studies to Evaluate Human Cancer Risk, Austin, TX, December 5-8, 1990.

Marker? Disease



Ciroussel, et al., Biochem. Pharmacol. 39:1109, 1990

Amount of Each Adduct Formed in Rats

Adduct	Relative Amount Formed in Liver	T½
OEdG	100	62 hr
EdC	0.3	>30 days
EdA	0.1	>30 days
EdG	1	>30 days

Swenberg *et al.*, "Progress in Predictive Toxicology" pp. 161-184, 1990. Elsevier Sci. Pub.

Persistence of Adducts in Liver

Rats exposed to 600ppm VC for 5 days

	Molar Concentration		
Adduct	Day 0	Day 7	Day 14
OEdG	1.6×10^{-4}	1.2×10^{-5}	—
EdG	1.8×10^{-6}	8.4×10^{-7}	4.7×10^{-7}
EdC	9.0×10^{-7}	7.0×10^{-7}	6.7×10^{-7}
EdA	1.8×10^{-7}	1.3×10^{-7}	0.8×10^{-7}

Swenberg *et al.* "Progress in Predictive Toxicology", pp. 161-184, 1990. Elsevier Sci. Pub.

Ability of Adducts to Induce Mutations

Adduct	Mutation Caused
OEdG	none
EdC	C→T
	C→A
EdA	A→T
	A→C
	A→G
EdG	G→A

Barbin, *et al.*, Cancer Res. 45: 2440, 1985
Barbin, *et al.*, Nucleic Acids Res. 9: 375, 1981
Hall, *et al.*, Carcinogenesis 2: 141, 1981
Spengler & Singer, Nucleic Acids Res. 9: 365, 1981

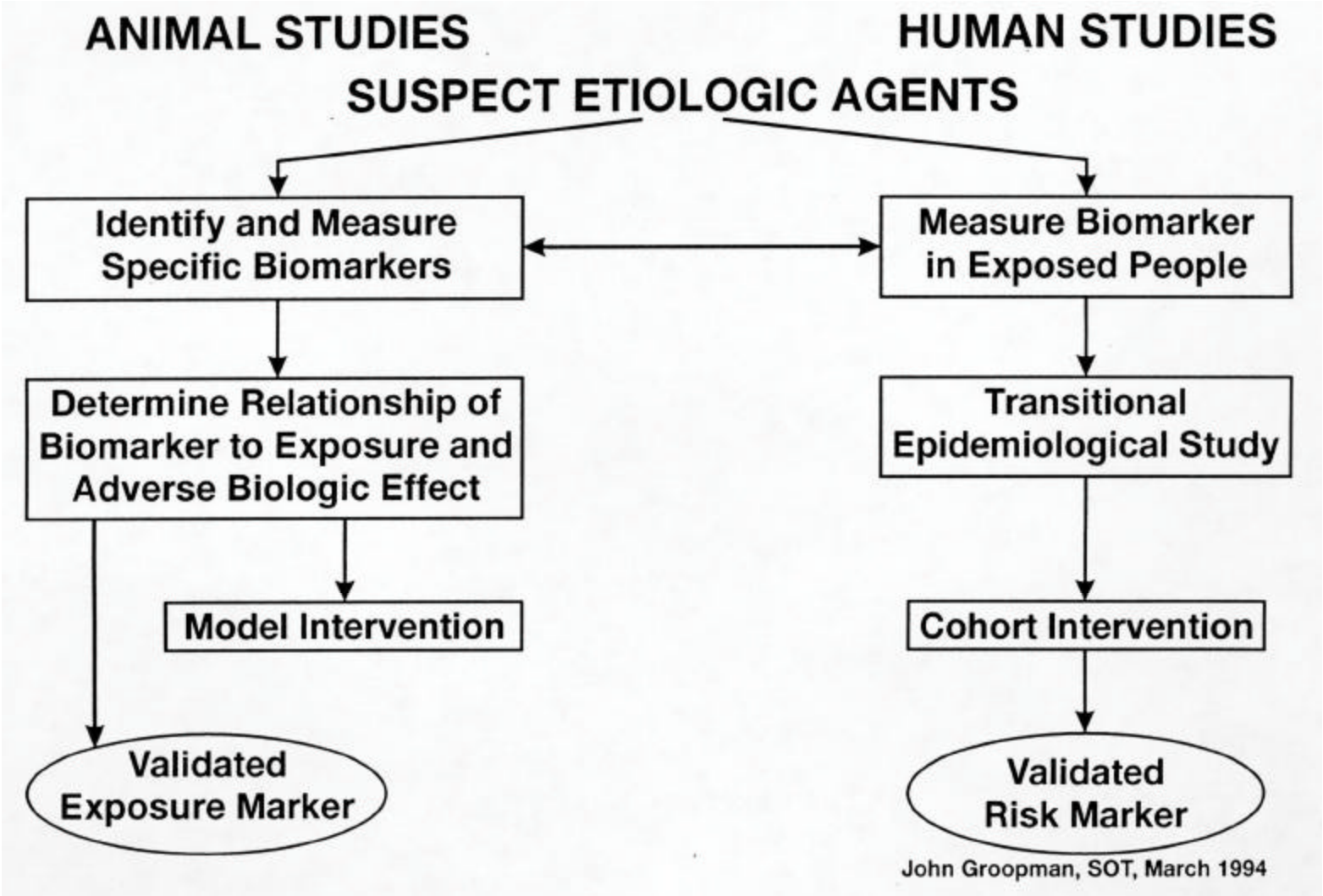
Relative Amount of VC Adducts in Tissues of Rats

Exposure: 600ppm VC, 4 hr/day for 5 days.

	Concentration ($M \times 10^{-6}$)				EdG / OEdG
	OEdG	EdG	EdA	EdC	
Newborn					
Liver	162	1.8	0.18	0.9	0.011
Lung	20	0.25	0.11	0.25	0.010
Kidney	29	0.31	ND	ND	0.011
Brain	ND	ND	0.06	0.22	–
Spleen	ND	ND			–
Adults					
Liver	43	0.47	0.19	0.80	0.011
Lung	20	0.27			0.014
Kidney	ND	ND			–

Fedtke *et al.*, Carcinogenesis 11: 1287-1292, 1990

The Validation Process: Confirming the Biomarker Disease Link



Conclusions

- **Biomarkers can be valuable for reducing uncertainties in assessing risk for disease from chemical exposures.**
- **More information is needed on mechanisms of disease induction by chemicals; such studies will suggest the most appropriate biomarkers of the biologically effective dose.**
- **Much research effort will be required to establish quantitative relationships between the level of markers present and both the degree of prior exposure and the predictability of health outcome.**

Criteria Air Pollutants

Two oxidizing agents: Ozone and Nitrogen Dioxide

Ozone

- 8-oxo-7,8-dihydroguanine (8-oxodG)

  Found in oral swabs, white blood cells and urine

  Urinary 8-oxodG results from DNA repair processes

  Nonspecific marker of oxidative stress

- Clara cell protein in serum

  Reported to be sensitive to as low as 0.060-0.084 ppm ozone

Criteria Air Pollutants (Con't)

Two oxidizing agents: Ozone and Nitrogen Dioxide

Nitrogen Dioxide

- Can form 8¢-nitroguanosine in RNA and 8-nitro-2¢-deoxyguanosine in DNA

  The latter is unstable

  The 8-nitroguaniosine has been used as a marker of <u>endogenous</u> reactive nitrogen species

- Urinary nitrate <u>does</u> <u>not</u> correlate with NO_2 exposure

- NO-heme (cannot distinguish between endogenous and exogenous reactive nitrogen)

NO_2 Disproportionates in Water

$$2\ NO_2 + H_2O \rightleftharpoons HNO_2 + HNO_3$$

$$2\ HNO_2 \rightleftharpoons NO + NO_2 + H_2O$$

$$NO + \text{heme protein} \rightleftharpoons NO/\text{heme protein}$$

NO/heme complex is detectable by its distinctive ESR signal

A Biological Marker of Exposure to SO_2:

S-Sulfonates in Nasal Lavage Fluid

$$SO_2 + H_2O \longrightarrow H^+ + HSO_3^-$$

$$HSO_3^- + -S\ -S \longrightarrow -S-SO_3^-$$

$$-S-SO_3^- \xrightarrow[OH]{CN^-} HSO_3^-$$

CO

Carboxy hemoglobin

Lead

Blood lead —— soft tissue lead

Urinary Pb
Plasma Pb
> reflect recent lead exposures

Bone Pb
Hair Pb
Teeth Pb
> reflect long term Pb exposures

H_2S

No biomarker of exposure found

Particulate Matter

- Carbon — elemental
  - organic (including PAH)
- Metals

Markers

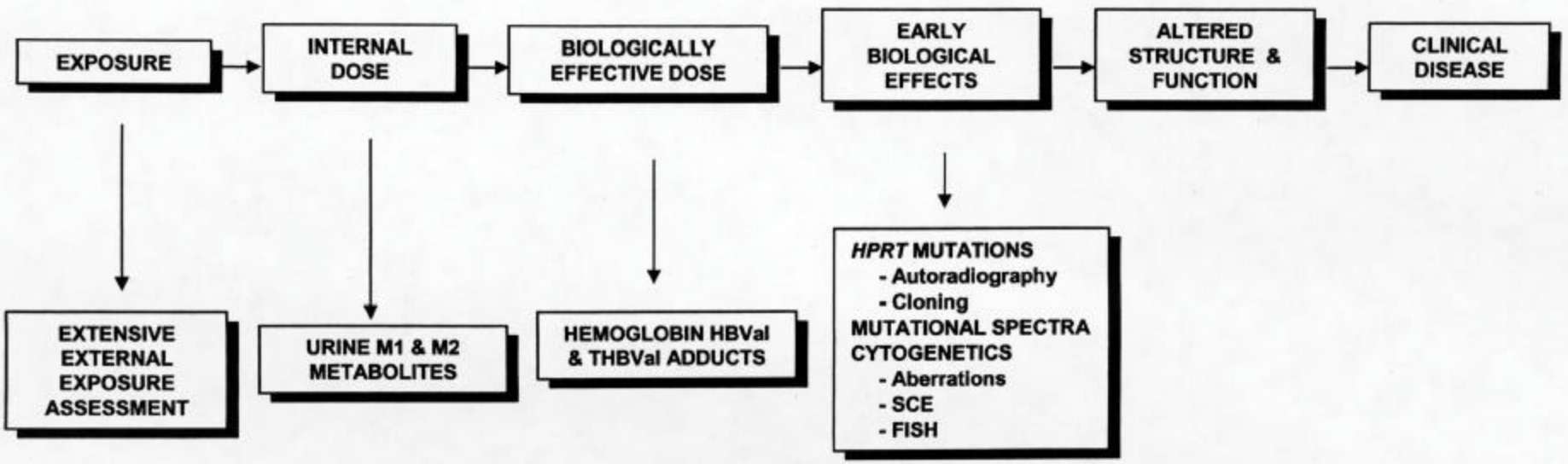
- 8-oxod G (in WBC)
- 1-hydroxy pyrene
- Fibrinogen
- Platelet counts
- Total WBC
- Particles in sputum macrophages

Air Toxics

VOCs

- Offer many opportunities for chemical-specific biomarkers of exposures:
  - Parent compound or metabolites in blood, urine or exhaled breath
  - DNA or protein adducts
- Studies by Albertini on 1,3-butadiene illustrate good transitional study

BIOMARKER RESPONSES IN BUTADIENE- EXPOSED CZECH WORKERS: A TRANSITIONAL EPIDEMIOLOGICAL STUDY

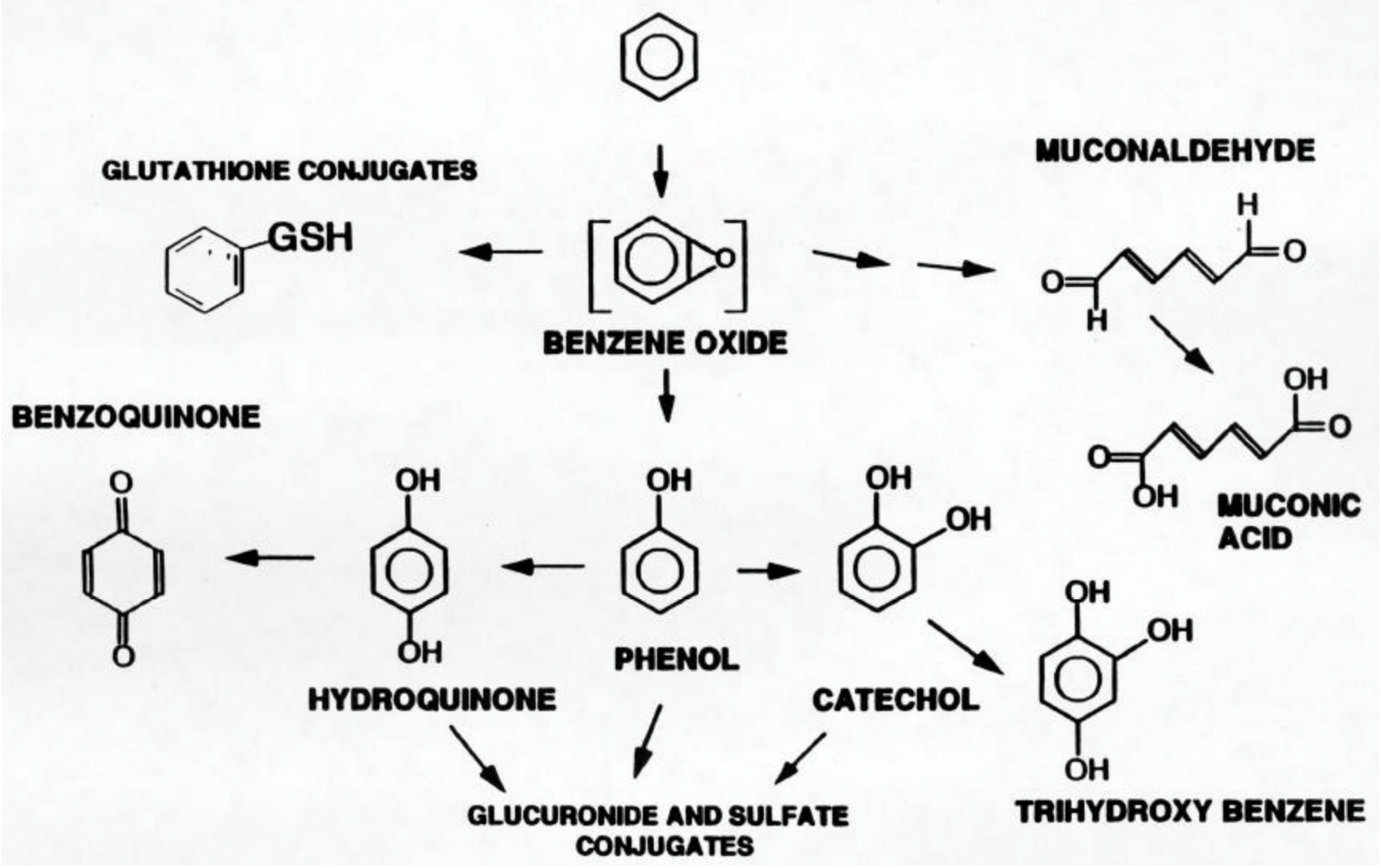


DIRECTION OF VALIDATION

M_1 = 1,2-Dihydroxy-4-(N-acetylcysteinyl)-Butene
M_2 = 1-Hydroxy-2-(N-Acetylcysteinyl)-3-Butane
HBVal = N-(2-Hydroxy-3-Butenyl)Valine
THBVal = N-(2,3,4-Trihydroxybutyl)Valine
FISH = Fluorescence *in situ* Hybridization

CYP2E1 = A P450 Enzyme
GST = Glutathione-S-Transferase
EH = Epoxide Hydrolase
ADH = Alcohol Dehydrogenase

Metabolic Pathway for Benzene



- **Metals**
  - **Markers of exposure are the metals in blood, urine, hair and toe or fingernails.**
- **PAHs**
  - **Urinary 1-hydroxypyrene**
  - **Total bulks DNA adducts by ^{32}P-postlabeling technique**
- **Dioxin-like compounds**
  - **Compounds in fat**
- **Mixtures**
  - **Need signature markers for specific sources of pollutants**

- **Proteomics and Genomics**
  - **Exciting new tools that may provide excellent biomarkers in the future**
  - **Currently we are still learning how to interpret our findings**

- **Sensitive Subpopulations**
  - **Polymorphisms can be valuable biomarkers**
  - **Examples:**
    - **NA D(P)H: quinone oxidoreductase: enzyme responsible for reducing reactive quinones to less reactive hydroquinones**
    - **Glutathione-S-transferase M1: enzyme required for detoxication of many electrophilic metabolites**
    - **DNA repairs enzyme**